

Electronic appendix:

Accompanying information to the article by Hailer *et al.*

“Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline”

Material and methods

Sample collection and DNA extraction

Blood samples were taken in the field from the brachial vein, immediately buffered in EDTA/SSC buffer and kept frozen until treatment in the lab. DNA extraction followed a standard protocol involving digestion with proteinase K and extraction with phenol-chloroform (Sambrook *et al.* 1989). Additionally, some naturally shed feathers were collected in the field. DNA from those was extracted using the DNeasy Tissue Kit (Qiagen) following the recommendations of Horváth *et al.* (2005). Our sampling within populations aimed at maximising the number of different territories, but never picking more than one sample per territory. We thus obtained a data set of presumably unrelated individuals, at least in the current generation. Spatially, our sampling in Estonia covers both the eastern and western distribution range, in Germany both northern and southern regions (Mecklenburg-Vorpommern and Brandenburg), in Sweden basically the entire distribution range, and the Norwegian samples stem from four different regions along the Atlantic coast (Møre-Romsdal, Sør-Trøndelag, Nord-Trøndelag and Troms).

PCR amplification and analysis of microsatellite markers

Fourteen loci cloned from the white-tailed eagle (*Hal 01* to *Hal 10* and *Hal 12* to *Hal 15*) were genotyped as described in Hailer *et al.* (2005). Additionally, 12 microsatellite markers developed for other raptor species were analysed in five multiplex reactions: *Aa35* (Martinez-Cruz *et al.* 2003), *Hle0B06*, *Hle0B10*, *Hle6A09*, *Hle6H10*, *Hle0E05*, *Hle0E12*, *Hle6F02*, *Hvo59* (Tingay *et al.*, *in prep.*), *IEAAAG04*, *IEAAAG05*, *IEAAAG14* (Busch *et al.* 2005). PCR reactions of the latter markers were performed in reaction volumes of 10 µL containing 10 ng of genomic DNA, 0.2 mM of each dNTP, 0.125-0.8 µM (see table S1) of each forward and reverse primer (one of them fluorescently labelled), 0.4 units of HotStarTaq DNA polymerase (Qiagen) and 1 µl of 10x HotStarTaq (Qiagen) reaction buffer containing Tris-Cl, KCl, (NH₄)₂SO₄ and a final concentration of 1.5 mM MgCl₂. We used the following PCR programme on a PTC-225 machine (MJ Research): 35 cycles with 95 °C for 30 sec., a locus-

specific annealing temperature (see Table S1) for 30 sec., and 72 °C for 30 sec. Before the first cycle, a prolonged denaturation step (95 °C for 15 min.) was included and the last cycle was followed by an additional annealing step at the corresponding annealing temperature for one minute and a final extension step for 8 min. at 72 °C.

PCR products were run on a MegaBACE 1000 capillary sequencer (Amersham Biosciences) and analyzed using the software GENETIC PROFILER 2.0. The MICROSATELLITE TOOLKIT for Excel (Park 2001) was used to calculate Nei's unbiased estimate of expected heterozygosity (Nei 1978), observed heterozygosity and mean number of alleles per locus. Deviation from Hardy-Weinberg equilibrium (HWE) was tested globally and separately for each locus in each population using the exact test implemented in GENEPOP 3.4 (Raymond and Rousset 1995). Theta, an estimator of F_{ST} (Weir & Cockerham 1984) was calculated using GENETIX (Belkhir *et al.* 2004) and its 95% confidence intervals were estimated by bootstrapping across loci 1000 times. Assignment tests were carried out using GENECLASS 2.0.d (Piry *et al.* 2004), employing the frequency-based method described in Rannala and Mountain (1997). As input data for the starting point of the demographic simulations in BOTTLESIM, we used all 26 loci from the Swedish (SWE) population. Simulations were performed assuming a life span of 17 years, sexual maturity at 5 years of age and fully overlapping generations (Helander 2003; Struwe-Juhl 2003). In BOTTLESIM, fertility and survival rates are held constant across each individual's life span.

Amplification and analysis of mtDNA control region sequences

We designed primers flanking the mitochondrial DNA control region using the published sequence of the common buzzard (*Buteo buteo*, GenBank accession number NC 003128). Primers *Bbu14834F* (5'-GGTCTTGTAACCAAAACTGAAGGC-3') and *Bbu16634R* (5'-CGGTTTAGGGGAGTCAGAGAGTAGTTTAA-3') were initially used to amplify the complete mtDNA control region in a few individuals of different geographic origin. Next, an especially variable 544 bp region was targeted by designing interior primers specific to *H. albicilla*: *HalHVR1F* (5'-CCCCCCTATGTATTATTGT-3') and *HalHVR1R* (5'-TCTCAGTGAAGAGCGAGAGA-3'). PCR reactions were carried out in 10 µl volumes containing approximately 15 ng of genomic DNA, 0.3 µM of each primer, 0.2 mM of each dNTP, 0.25 units of HotStarTaq DNA polymerase (Qiagen) and 1 µl of 10x HotStarTaq (Qiagen) reaction buffer containing Tris-Cl, KCl, (NH₄)₂SO₄ and a final concentration of 1.5 mM MgCl₂. PCR was performed in a PTC-225 instrument with the following treatment: 15 min. at 95 °C prior to 36 cycles of 30 sec. at 56 °C, 30 sec. at 72 °C and 30 sec. at 95 °C.

Finally, a 1 min. step at 56 °C and an extension step of 10 min. at 72 °C was performed. PCR products were cleaned using the ExoSAP enzyme kit (Amersham Biosciences) and DNA sequencing was performed on both strands using the original PCR primers and the DYEnamic ET Terminator kit (Amersham Biosciences). Sequencing reactions were cleaned using AutoSeq plates (Amersham Biosciences) and run on a MegaBACE 1000 capillary instrument according to the manufacturer's recommendations. Electropherograms were checked manually and assembled in Sequencher 4.1.4 (Gene Codes). After removal of primer sequences and some additional bases close to the primers, this yielded a 500 bp fragment for analysis.

Bootstrap resampling to standardize estimates of genetic diversity for sample size

For each population, we used the Excel macro POPTOOLS (Hood 2005) to randomly resample individuals with replacement, creating 100 synthetic populations of equal size: 10 individuals for both the microsatellite and mtDNA analysis, corresponding to the number of samples in the smallest population sample (Kola peninsula). Then, for the microsatellite data, the macro MICROSATELLITE TOOLKIT for Excel (Park 2001) was used to calculate the unbiased expected heterozygosity, observed heterozygosity and mean number of alleles per locus for each of the synthetic populations. The average of these 100 values is given in Table 1. In order to calculate the corrected number of mitochondrial DNA (mtDNA) haplotypes, basically the same procedure was used. After bootstrap resampling of individuals, each haplotype was coded as a number and the data was then analyzed as haploid genotype data using MICROSATELLITE TOOLKIT. From that output, we determined the number of haplotypes per synthetic population by counting all haplotypes with a frequency larger than zero. The average number of haplotypes among the 100 replicates is given in Table 1.

Distribution map

Information regarding the distribution of white-tailed eagles was obtained from Folkestad (*unpublished data*) and Ganusevich (*unpublished data*), Hauff (1998), Hauff (*unpublished data*), Helander *et al.* (2003), Mizera (2002), Randla (1976) and Stjernberg (*personal communication*).

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Table S1: Multiplex assays for 12 microsatellites cross-species amplified in the white-tailed eagle.

Multiplex	annealing temperature (°C)	marker	concentration (μM)
1	52	<i>Aa35</i> (F)	0.35
		<i>Aa35</i> (R)	0.35
		<i>Hle0B06</i> (F)	0.30
		<i>Hle0B06</i> (R)	0.30
		<i>Hle0B10</i> (F)	0.55
		<i>Hle0B10</i> (R)	0.55
2	52	<i>Hle6A09</i> (R)	0.35
		<i>Hle6A09</i> (F)	0.35
		<i>Hle6H10</i> (F)	0.45
		<i>Hle6H10</i> (R)	0.45
3	52	<i>Hle0E12</i> (F)	0.65
		<i>Hle0E12</i> (R)	0.65
		<i>HleE05</i> (F)	0.80
		<i>HleE05</i> (R)	0.80
4	54	<i>Hvo59</i> (F)	0.50
		<i>Hvo59</i> (R)	0.50
		<i>Hle6F02</i> (F)	0.50
		<i>Hle6F02</i> (R)	0.50
5	56	IEAAAG04(F)	0.125
		IEAAAG04(R)	0.125
		IEAAAG05(F)	0.125
		IEAAAG05(R)	0.125
		IEAAAG14(F)	0.50
		IEAAAG14(R)	0.50